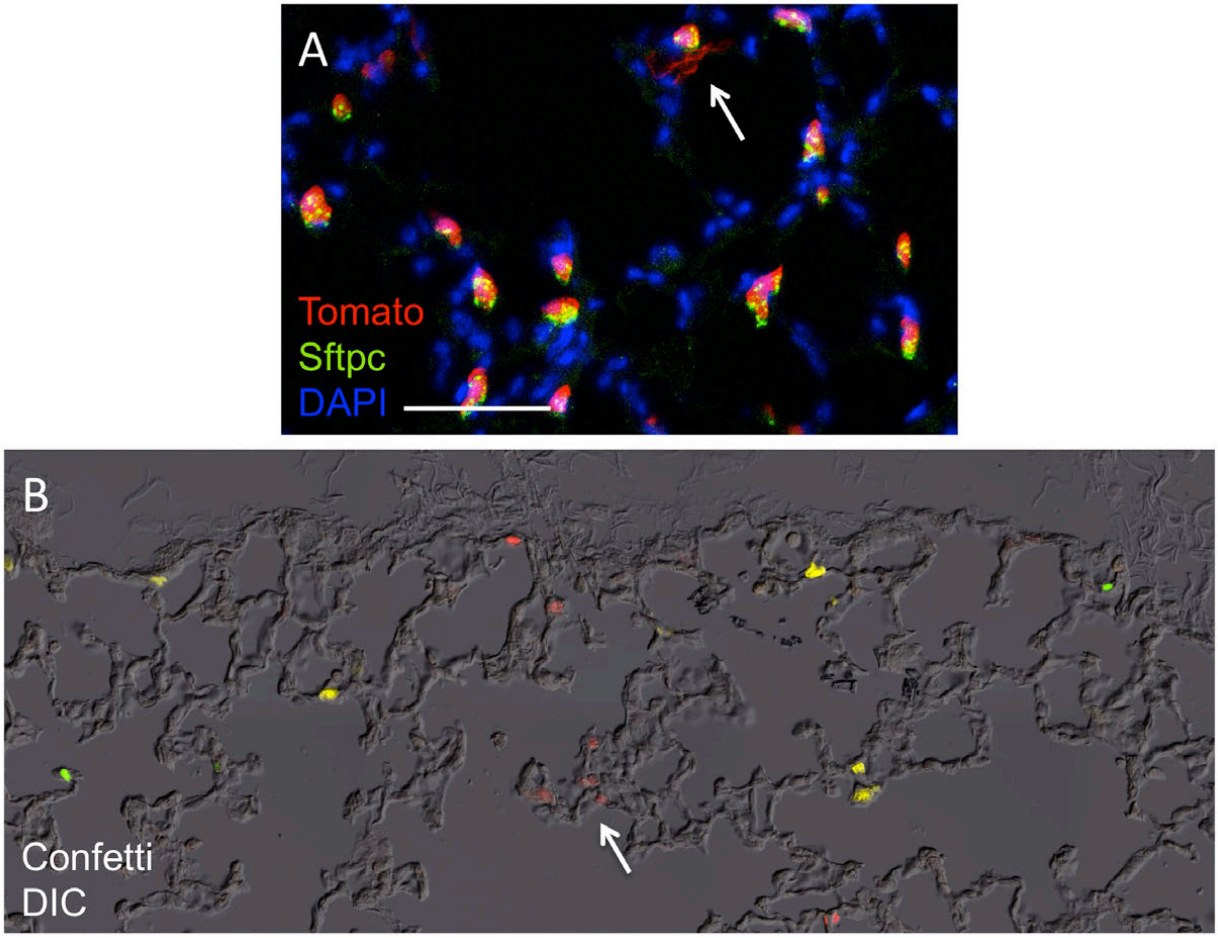
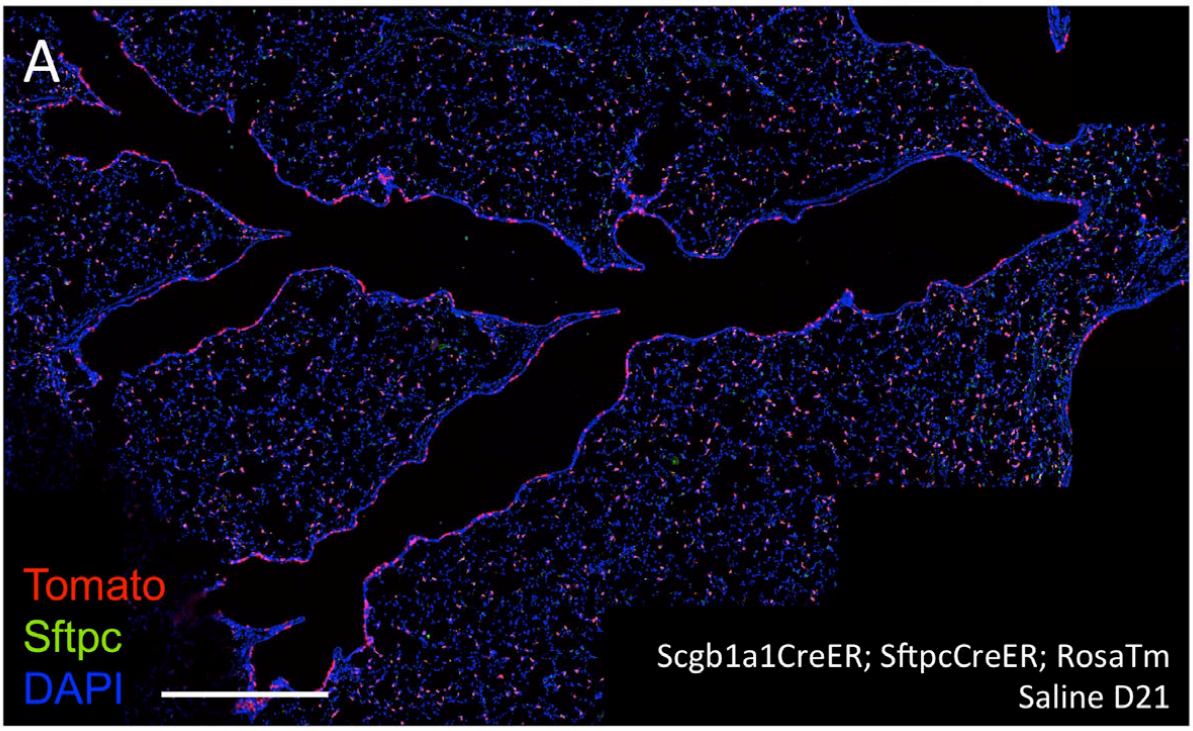
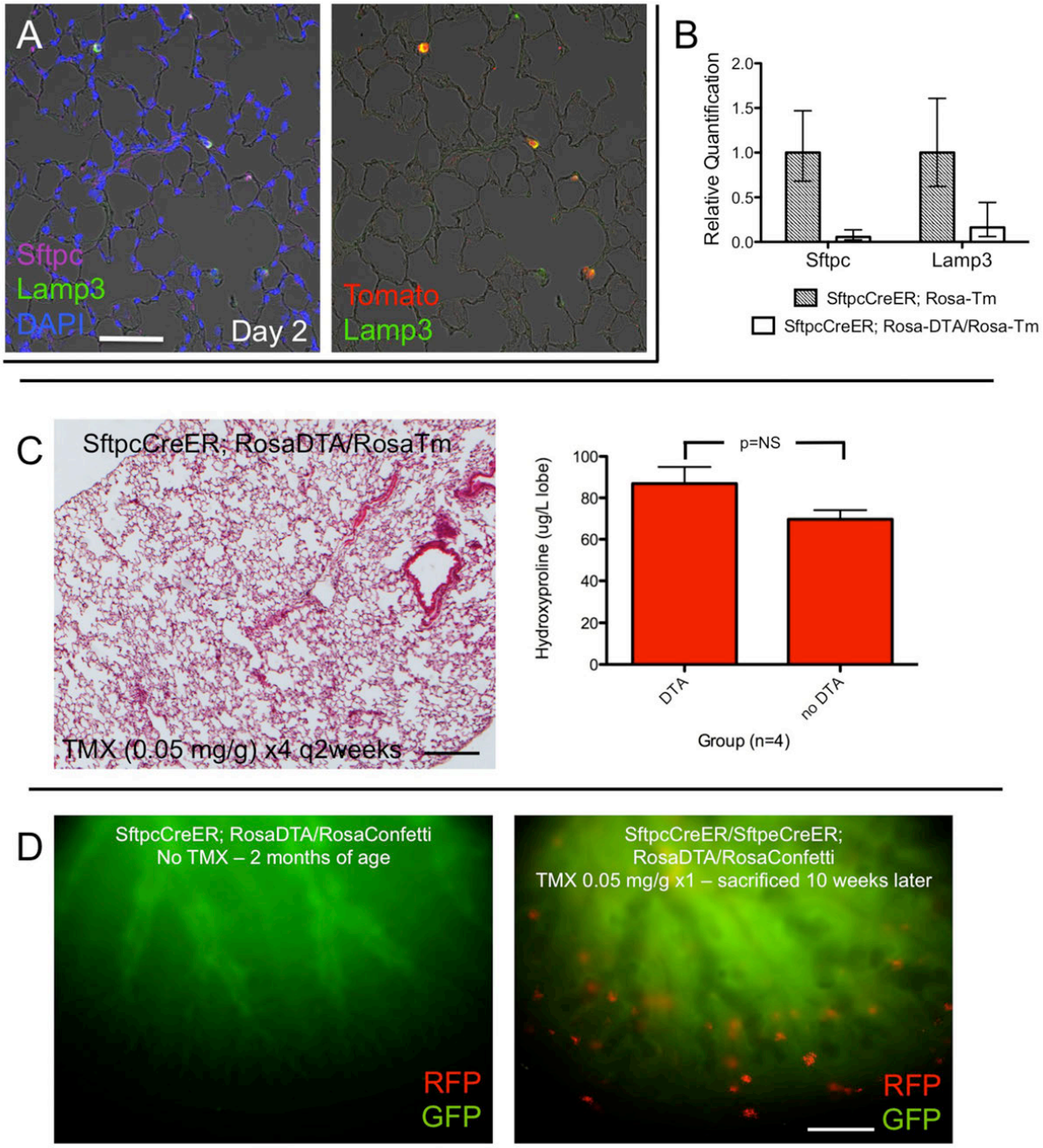


Supplemental Figure S1, Relevant to Figure 1. Lineage-labeled AEC1s at steady-state and only small clones are generated by AEC2s in the adult lung in the absence of experimentally induced repair.

(A) Sections from an SftpcCreER; Rosa-Tm mouse described in Fig. 1C, 24 weeks after Tmx injections, showing lineage-labeled AEC1s (arrow) that have arisen at steady-state. (B) Adult Sftpc-CreER; Rosa-Confetti mice were dosed once with 0.1 mg/g Tmx. After 7 months (28 weeks), tiled confocal DIC images of lung frozen sections show cells labeled with nuclear GFP (nGFP), cytoplasmic YFP, membrane cyan (mCFP) and cytoplasmic RFP. Most cells are single but some small clusters are present (arrow) showing that clonal expansion is minimal in the absence of repair promoted by DTA-induced AEC2 death. Scale bar: (A) 50μm.



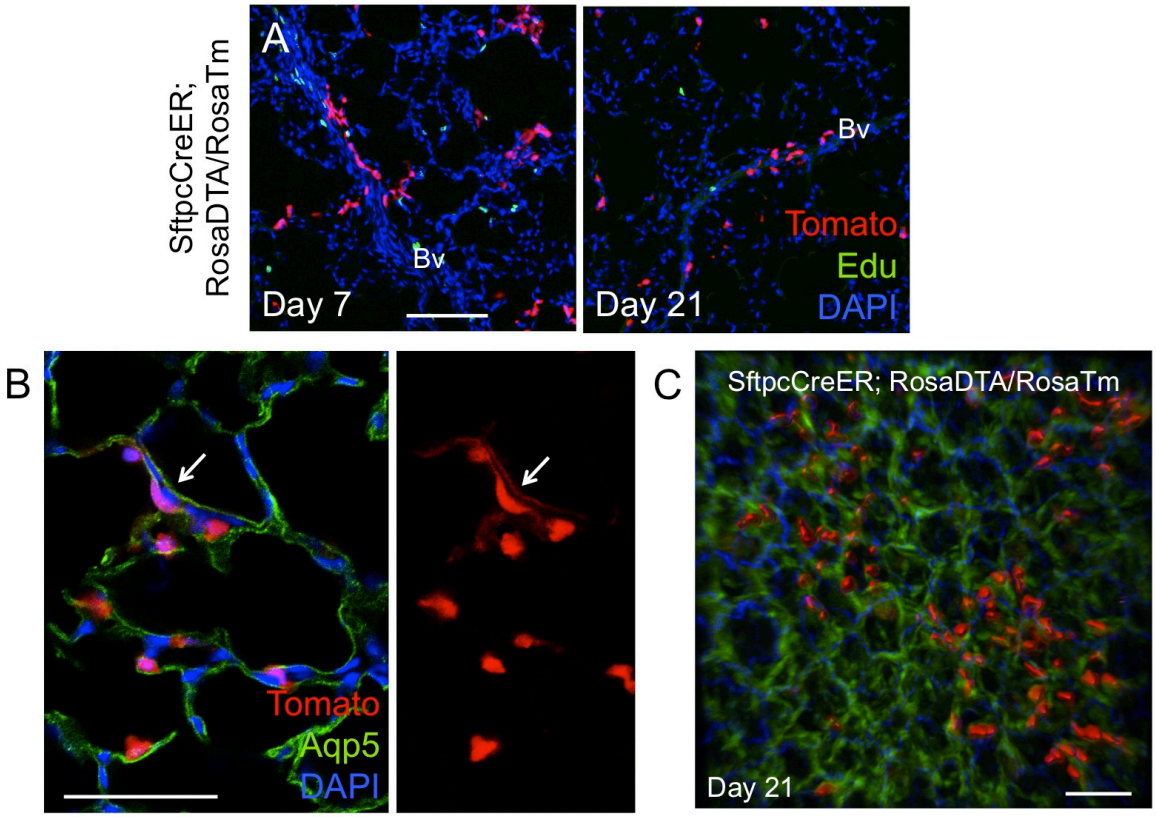
Supplemental Figure S2, Relevant to Figure 2. Extensive lineage labeling of both airway and alveolar cells in lungs with both *Sftpc-CreER* and *Scgb1a1-CreER* alleles.
(A) *Sftpc-CreEr; Scgb1a1-CreER; Rosa-Tm* mice were dosed x4 with 0.2 mg/g Tmx and then with saline (as a control for bleomycin treatment). Lungs were harvested 21 days later and sections stained with antibody to Sftpc (with instrinsic Tm present as well). A representative tiled confocal image demonstrates efficient recombination in both airway epithelium and alveolar AEC2s (compare with Figure 1A). Scale bar: (A)500μm.



Supplemental Figure S3, Relevant to Figure 3. Sftpc and Lamp3 expression after DTA ablation; Absence of fibrosis following DTA-induced loss of AEC2s; Absence of spontaneous lineage-labeled clusters in *SftpcCreER; Rosa-Confetti* mice.

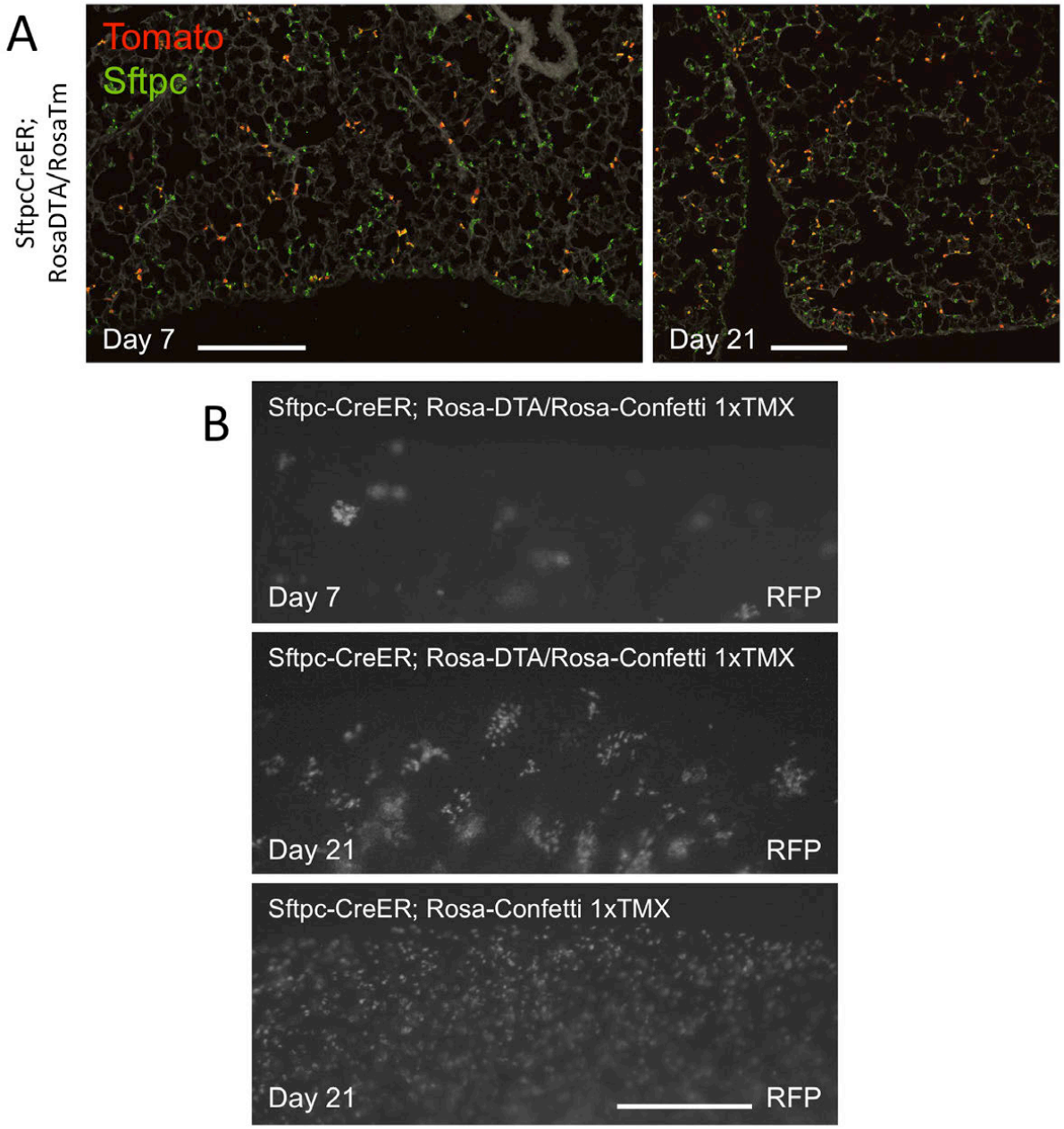
(A) Frozen sections from *SftpcCreER; Rosa-DTA/Rosa-Tm* mice sacrificed 2 days after Tmx were stained for AEC2 makers Sftpc and Lamp3. There is a decrease in numbers of Sftpc and Lamp3 expressing AEC2s following DTA ablation. These AEC2 markers co-localize. (B) qRT-PCR confirms expression of AEC2 markers is decreased following DTA ablation (n=5 controls; n=6 DTA mice; error bars represent 95% confidence interval). (C) Cohorts (n=4) of *Sftpc-CreER; Rosa-DTA/Rosa-Tm* and *SftpcCreER; Rosa-Tm* mice were given four doses of Tmx (0.05mg/g), each 2 weeks apart. 2 weeks later lungs were harvested and sections stained with hematoxylin and eosin. Note absence of fibrosis and essentially complete repair of lung architecture in lungs with the *Rosa-DTA* allele. Hydroxyproline content assayed to estimate collagen content (n=4

mice per group). There is no significant difference between the control and experimental groups. Error bars are mean +/- SEM. (D) Non-Tmx-treated *SftpcCreER; Rosa-DTA/Rosa-Confetti* mice (n=3) were sacrificed between 8 and 9 weeks of age and tissue examined after clearing in Scal*e* and imaging for RFP and GFP on fluorescent stereoscope (left panel). Compare to Tmx-treated *SftpcCreER/SftpcCreER; Rosa-DTA/Rosa-Confetti* animal 10 weeks after injection (right panel). Imaging reveals no evidence of clusters of lineage-labeled AEC2s in absence of Tmx. GFP reflects the endogenous GFP in the Rosa-DTA construct. Scale bars: (A) 50μm; (C)100μm; (D)500μm.

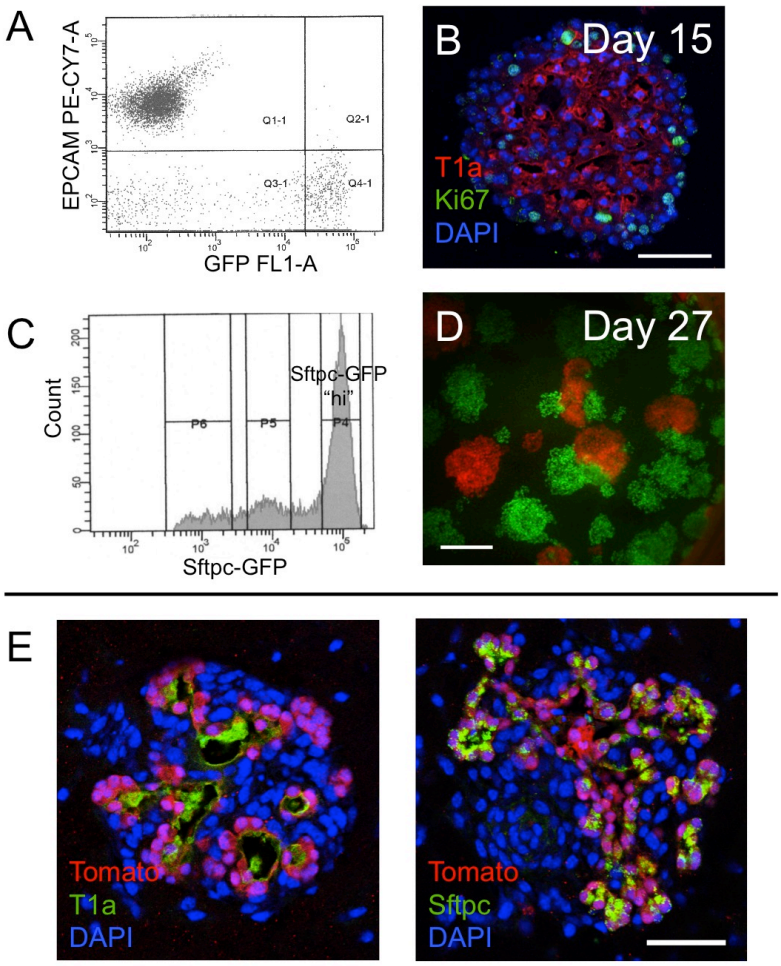


Supplemental Figure S4, Relevant to Figure 4. Linear arrangement of some clones in alveolar region undergoing repair following DTA-induced AEC2 death; Lineage-labeled AEC1; Multiphoton image of lungs 21d after DTA injury.

(A) Samples are from the experiment depicted in Fig.4A, Upper panels. At 7 and 21 dpi, cells making up some clones are present in a linear arrangement along what appears to be a blood vessel (Bv). (B) Sample from experiment depicted in Fig.4A, upper panels, 21 dpi. Lineage-labeled AEC1 expresses Aqp5 (arrow). (C) Sample is from the experiment depicted in Figure 4B. At 21 dpi, cells making up clones appear more dispersed, with greater numbers of Tm+ cells than at 7d. Scale bars: (A)100μm; (B,C)50μm.

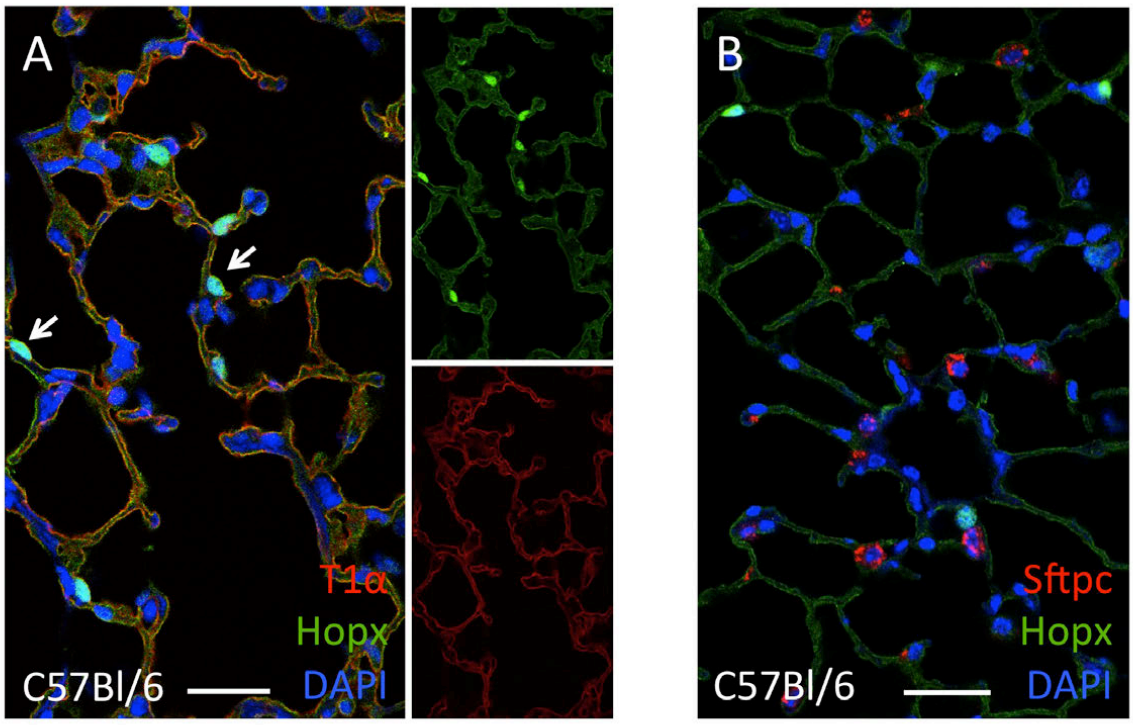


Supplemental Figure S5, Relevant to Figure 4. Normal Sftpc expression is restored by 21d after DTA injury; Clonal proliferation after DTA injury.
(A) Tiled images from samples described in Fig. 4C demonstrate uneven distribution of Sftpc staining at 7 dpi in Sftpc-CreER; Rosa-DTA/Rosa-Tm animals. By 21 dpi, Sftpc distribution appears normal and repair is complete. (B) Findings depicted in Fig. 4B,S4B are recapitulated in Sftpc-CreER; Rosa-DTA/Rosa-Confetti animals at 7 and 21 dpi. Scale bars: (A)200μm; (B)500μm.



Supplemental Figure S6 Relevant to Figure 5. Characterization of Pdgfra-GFP+ cells; Proliferation, self-renewal, CFE and clonal growth of Sftpc+ lineage labeled cells in 3D culture; AEC2s grown with MLg cells.

(A) *Pdgfra-H2B:GFP+* mouse lung was dissociated and a single-cell suspension was stained for CD31 (endothelial cells), CD45 (hematopoietic cells), and EpCAM (epithelial cells). A non-endothelial, non-hematopoietic, non-epithelial "stromal" cell population was sorted by gating against dead cells, CD31+, CD45+, and EpCAM+. Of this population, 28.8% of the cells were Pdgfra-GFP+. This suggests that less than one third of the mesenchymal cells in the lung are Pdgfra+. (B) Day 15 sphere with Ki67+ cells in periphery, suggesting that these cells are proliferating. Note that the differentiated T1a (podoplanin) positive AEC1s in the interior of the sphere are negative for Ki67. (C,D) Evidence that spheres are formed from single cells and not by cell–cell aggregation. *SftpcCreER; Rosa-Tm* mice were given 4 doses of Tmx and Tomato+ cells isolated by FACS. AEC2s were also isolated from Sftpc-GFP transgenic mice (GPF "hi") (C). Equal numbers of Tm+ and GFP+ cells were mixed and grown in 3D culture as described. Throughout growth, no evidence was seen for early aggregation of Tm+ and GFP+ cells. Rather, the discrete Tm+ and GFP+ colonies observed at Day 27 (D) suggest that spheres form from single cells. (E) Representative colonies from AEC2s plated with MLg fibroblasts. Note dense clustering of MLg cells around outside of epithelial cells. Scale bars: (B) 50μm (D)200μm; (E)50μm.



Supplemental Figure S7 Relevant to Figure 5, Immunohistochemistry for Hopx in normal mouse lung. (A) Hopx and T1a staining appear to overlap, highlighting AEC1s. Hopx antibody also stains scattered nuclei, presumably nuclei of AEC1s based upon location on surface of alveolar structure (arrows). (B) There is no apparent colocalization of Sftpc and Hopx staining in the mouse lung. Scale bars: (A,B)25μm.

Supplemental Table 1. Primers used in qRT-PCR (Fig. S3D)

Gene	Forward	Reverse
Gapdh	5’-AGGTCGGTGTGAACGGATTTG-3’	5’-TGTAGACCATGTAGTTGAGGTCA-3’
Sftpc	5’-CAAACGCCTTCTCATCGTGGTTGT-3’	5’-TTTCTGAGTTTCCGGTGCTCCGAT-3’
Lamp3	5’-TCCAAAAGCCAGAGGCTATCT-3’	5’-ACTGGGGTTACTGTTTTCATTGT-3’